Purification

Collagen was precipitated from the supernatant liquid by adding 140 g NaCl with stirring and allowing the liquid to stand for 4 hours at 5°C. The precipitated collagen was centrifuged out at 30,000 g for 30 minutes. The resulting collagen pellet was taken up in 200 ml distilled water and 0.5 N acetic acid was added to make one liter. The collagen was precipitated from this solution by adding 50 g NaCl, allowing the solution to stand for 4 hours at 5°C and centrifuging at 30,000 g for 30 minutes.

Sterilization

The resulting collagen pellet was taken up in 200 ml distilled water, placed in sterilized dialysis tubing and dialysed for 72 hours against 50 volumes 1 N acetic acid. Acetic acid concentration was reduced by then dialysing the solution twice for 24 hours against 50 volumes 0.001 N acetic acid. The solution was then concentrated by placing the dialysis tube on sterile absorbant towels in a laminar-flow bacteriologic barrier until the concentration reached 12–15 mg collagen/ml solution. A known pH was then reestablished by dialysing the concentrate against 50 volumes 0.001 N acetic acid for 24 hours. Following this the concentrate was stored in sterile vials at 5°C pending use.

Addition of Polymerization Promoter to Concentrate

Just prior to use a buffered salt solution, NaCl 2.5 mM/l, $NaHPO_4$ 0.1 mM/l, pH7.4, was added at 5°C to the concentrate in a volume: volume ratio of 1:10 (buffer:collagen), and the buffered concentrate was transferred to a chilled (5°C) syringe.

Use

The above described collagen solution was successfully used as a soft tissue augmentation material in rabbit to rabbit and rabbit to rat implants. The solution polymerized in situ as described above.

EXAMPLE 2

An injectable solution of rat collagen was prepared by the procedure of Example 1 and used successfully as a soft tissue augmentation material in rat to rat implants.

EXAMPLE 3

An injectable solution of human collagen was prepared by the procedure of Example 1 and used successfully as a soft tissue augmentation material in human to rabbit and human to rat implants.

Modifications of the above described invention and the materials and procedures used to make the same which are employed in the invention which are obvious to persons of skill in the biochemical and/or medical arts are intended to be within the scope of the following claims.

We claim:

1. Method of augmenting connective tissue in a living mammal comprising administering a solution of solubilized, purified, native, in situ polymerizable collagen to the mammal at the augmentation site which polymerizes at said site into a fibrous mass of tissue.

2. The method of claim **1** in which the solution is implanted into the mammal by injection.

3. The method of claim **1** in which the solution is administered by coating the augmentation site.

4. The method of claim **1** in which the collagen source is heterologous skin or tendon.

5. The method of claim **1** in which the mammal is a human.

6. The method of claim **1** in which the concentration of collagen in the solution is up to 20 mg/ml.

7. The method of claim **1** in which the concentration of collagen in the solution is about 12 to about 15 mg/ml.

8. The method of claim **1** in which the pH of the solution is about 6 to about 8.

9. The method of claim **1** in which the solution contains a polymerization promoter which causes the solution to be isotonic.

10. The method of claim **9** in which the promoter is a buffered, neutral salt solution.

11. The method of claim **10** in which the buffered, neutral salt solution is a solution of NaCl and $NaHPO_4$, pH 7.4.

12. The method of claim **1** in which the collagen has been solubilized by treatment with a proteolytic enzyme.

13. The method of claim **12** in which the treatment with a proteolytic enzyme is made in an acid medium, pH of 1 to 4.5 at a temperature in the range of 0°C to 15°C.

14. The method of claim **13** in which the temperature is 11°C.

15. The method of claim **14** in which the proteolytic enzyme is pepsin.

16. The method of claim **1** in which the solubilized collagen has been purified by salt precipitation.

17. The method of claim **9** in which the temperature of the solution is kept below about 5°C until it is administered.

18. The method of claim **1** in which particles of insoluble collagen microfibrils are added to the solution before it is administered.

19. The method of claim **1** in which the solution is administered to the skin as a sealer to cover a burn or an abrasion.

20. The method of claim **19** in which the solution contains a drug to prevent secondary infection.

21. The method of claim **1** in which the collagen has been solubilized by treatment with a proteolytic enzyme, the concentration of collagen in the solution is up to 20 mg/ml, the pH of the solution is about 6 to about 8 and the solution contains a polymerization promoter which causes the solution to be isotonic.

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